FULL PAPER
THE ANALYST
www.rsc.org/analyst

Microwave treatment of biological samples for methylmercury determination by high performance liquid chromatography–cold vapour atomic fluorescence spectrometry

Elsa Ramalhosa,*[a] S. Río Segade,[b] Eduarda Pereira,[c] Carlos Vale[d] and Armando Duarte[c]

[a] *Departamento de Agro-Indústrias, Escola Superior Agrária, Instituto Politécnico de Bragança, 5300 Bragança, Portugal. E-mail: elsa@ipb.pt*
[b] *Departamento de Química Analítica y Alimentaria, Universidad de Vigo, Facultad de Ciencias de Ourense, 32004 Ourense, Spain*
[c] *Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal*
[d] *Instituto de Investigação das Pescas e do Mar (IPIMAR), Av. Brasília, 1400 Lisbon, Portugal*

Received 8th May 2001, Accepted 20th June 2001
First published as an Advance Article on the web 14th August 2001

A simple and rapid microwave-assisted alkaline digestion procedure was developed in combination with high performance liquid chromatography–ultraviolet post-column oxidation–cold vapour atomic fluorescence spectrometric detection for methylmercury determination in biological tissues. Since the stability of methylmercury in methanolic potassium hydroxide solution under microwave irradiation was verified, the microwave-assisted extraction procedure was optimized in terms of quantitative recovery of methylmercury and minimum time required. The alkaline extracts were subjected to clean-up steps with dichloromethane and hydrochloric acid in order to reduce matrix interferences in methylmercury determination. The effects of matrix interferences were checked by comparison of the slopes corresponding to calibration and standard addition curves. The accuracy of the method was evaluated by the analysis of two biological certified reference materials, NRC TORT-2 and BCR 463. The results obtained by the proposed method were in good agreement with the certified values of methylmercury concentration in both materials. The detection limit was 10 μg kg^{-1} and the relative standard deviation was < 8% for methylmercury concentrations ranging from 0.15 to 3.0 mg kg^{-1}.

Introduction

The presence of mercury species in aquatic food chains was recognized long ago as a major environmental pollution issue and health hazard for humans.[1] Inorganic mercury may be converted into the more toxic methylmercury by aquatic organisms and be bioaccumulated in the food chain. As a consequence, fish usually contain high levels of methylmercury, with increasing concentration in the tissue with the trophic level and age of the fish. Methylmercury usually represents more than 85% of the total mercury present in fish.[2] Mercury speciation in fish samples is required because of the poisoning risk associated with the consumption of fish contaminated with methylmercury.

The most widely used analytical methodology for mercury speciation determination in biological samples usually involves several steps, including extraction of mercury compounds and chromatographic separation of the different species followed by element specific detection. The extraction of methylmercury from a complex matrix is a limiting step because of incomplete extraction, species transformation, sample contamination or analyte losses that can be produced during sample treatment. Hence, the efficiency, precision and accuracy of the overall method are mostly associated with the sample preparation procedure.[3] The Westöö method,[4,5] based on successive extractions and back-extractions with organic solvents and an aqueous complexing agent, is the most commonly used procedure for the extraction of methylmercury from biological samples. Other isolation methods are also applied to the extraction of organomercury compounds from biological tissues, such as acid leaching or alkaline digestion using conventional heating sources[6–8] or using steam distillation,[9,10] ultrasonic energy[11–14] or supercritical fluid extraction[15,16] to decrease the extraction time. The main disadvantages of the above procedures are the low recoveries reported for methylmercury, the number of extraction steps involved, the time required and the amount of solvent consumed.

Microwave energy has recently been applied to the acceleration of methods for methylmercury extraction from biological samples. Tseng *et al.*[17] developed a rapid and simple microwave-assisted alkaline digestion method with 25% m/v tetramethylammonium hydroxide for speciation determination of inorganic mercury and methylmercury in biological tissues by gas chromatography–electrothermal atomic absorption spectrometry (GC-ETAAS). Quantitative recoveries were obtained for an applied power of 40–60 W and a heating time of 2–4 min. The same extractant (25% m/v tetramethylammonium hydroxide) was used by other workers for the acceleration of inorganic mercury and methylmercury extraction from biological tissues in a microwave field at 45 W for 2.5 min,[18] at 120 W for 20 min[19] and at 20 W for 20 min.[20] Both mercury species were subsequently determined by gas chromatography–microwave induced plasma atomic emission spectrometry (GC-MIP-AES), high performance liquid chromatography–atmospheric pressure

DOI: 10.1039/b104041n

ionization-mass spectrometry (HPLC-API-MS) and GC-MIP-AES, respectively. Vázquez *et al.*[21] described the use of toluene in hydrochloric acid medium as an extractant for the complete extraction of methylmercury from fish tissues. Quantitative recoveries were obtained when the slurries were subjected to microwave irradiation for 10 min. Methylmercury determination was carried out by gas chromatography with electron capture detection (GC-ECD).

The most common methods for mercury speciation in biological tissues are based on a derivatization reaction to form volatile mercury species, which are then separated by GC and detected with an element specific detection method such as MIP-AES,[18,20,22] AAS,[17,23] cold vapour atomic fluorescence spectrometry (CV-AFS)[6,24] or inductively coupled plasma mass spectrometry (ICP-MS).[11,25] Nevertheless, methods which rely on HPLC have also been used for mercury speciation in biological samples coupled to ICP-MS,[10,26] photometry,[27] cold vapour atomic absorption spectrometry (CV-AAS)[12,13,28] or CV-AFS.[29,30]

The purpose of this work was to develop a simple, rapid and reliable microwave-assisted alkaline digestion method which permits the complete extraction of mercury compounds from fish tissues. The microwave-assisted extraction method was optimized in order to achieve quantitative recovery for methylmercury in a minimum time using 25% m/v methanolic potassium hydroxide as extractant. Organomercury species were subsequently extracted with dichloromethane and back-extracted into ultra-pure water in order to clean up the alkaline extract. Methylmercury was determined in the aqueous extract by high performance liquid chromatography–ultraviolet post-column oxidation–cold vapour atomic fluorescence spectrometry (HPLC-UV-PCO-CV-AFS). The combination of an alkaline digestion procedure with an HPLC-CV-AFS system for methylmercury determination in biological tissues has not previously been reported in the literature. The behaviour of methylmercury in alkaline solution under a microwave field was also investigated in terms of how to preserve the analyte identity and avoid analyte losses. The overall methodology was validated by the analysis of two certified reference materials of fish tissues containing different amounts of methylmercury.

Experimental

Instrumentation

The chromatographic system used in this work was as described by Ramalhosa *et al.*[31] Briefly, the system included an HPLC pump module (Knauer, Berlin, Germany), a six-port injection valve (Rheodyne, Cotati, CA, USA) equipped with a 200 μl PEEK sample loop and a reversed-phase analytical column packed with Nucleosil ODS (RPC_{18}, 25 cm × 4.6 mm id, 5 μm film thickness). All separations were performed at laboratory temperature under isocratic conditions. The post-column oxidation system consisted of a UV-irradiation lamp (8 W, 254 nm) (Camag, Muttenz, Germany) surrounded by a 3 m coil. A reduction coil (2 m), two flow meters and a quartz gas–liquid separator cell (PS Analytical, Kemsing, Kent, UK) were used for mercury cold vapour generation. The solutions were introduced into the system by four-channel peristaltic pumps (Ismatec, Zürich, Switzerland) through Tygon tubes (R 3603). The mixing joints and both reaction coils were made of 0.50 mm id Teflon. The drying of the mercury vapour generated was carried out in a sulfuric acid trap (0 °C) connected to a calcium chloride trap (7 cm × 1 cm id). A PS Analytical Model 10.023 Merlin atomic fluorescence spectrometer was used as a mercury detector.

A CEM MDS-81D microwave oven (600 W maximum output) with glass tubes of 22 ml and Gilson (Villiers le Bel, France) shakers were used for sample preparation.

Reagents and reference materials

All standards and reagents were prepared in ultra-pure water produced in a Milli-Q Model 185 system. The chemicals were of analytical-reagent grade and mercury free and were used without further purification. The mobile phase was a mixture of methanol (liquid chromatography grade, Merck, Darmstadt, Germany) (5% v/v) and ultra-pure water, containing 0.01% v/v 2-mercaptoethanol (Merck) buffered at pH 5 with 0.06% v/v acetic acid (Merck) and 0.15% m/v ammonium acetate (Merck). The mobile phase was filtered through 0.2 μm membranes (NL 16, Schleicher & Schüll, Dassel, Germany) and de-gassed in an ultrasonic bath for 30 min prior to use.

The reducing agent, 3% m/v tin(II) chloride in 15% v/v hydrochloric acid, was prepared daily by the dissolution of the appropriate amount of mercury-free tin(II) chloride (Merck) in mercury-free hydrochloric acid (Merck) on a hot-plate. The solution was diluted to volume with ultra-pure water, filtered through 0.45 μm membranes (Millipore, Bedford, MA, USA) and purified from mercury by bubbling with nitrogen for 2 h.

Stock standard solutions of mercury nitrate (1000 mg l^{-1}, Spectrosol, BDH, Poole, Dorset, UK) and methylmercury chloride (1000 mg l^{-1} in mercury, Alpha Products, Karlsruhe, Germany) were used weekly to prepare a working standard solution of 10 mg l^{-1} (as Hg) of each individual species in ultra-pure water. Lower concentration working standard solutions were prepared daily in ultra-pure water. They were stored under refrigeration at 4 °C.

A solution of 25% m/v methanolic potassium hydroxide was prepared daily by the dissolution of potassium hydroxide (Merck) in methanol (liquid chromatography grade, Merck). Dichloromethane was obtained from Merck (liquid chromatography grade).

Two biological reference materials with different certified contents of methylmercury were used to validate the proposed method. BCR 463 (Tuna Fish) was obtained from the Community Bureau of Reference (BCR) and TORT-2 (Lobster Hepatopancreas) was obtained from the National Research Council of Canada (NRC). The biological extracts were filtered through 0.2 μm membranes (PVDF PP, LIDA, Kenosha, USA) prior to injection.

Figures of merit

The analytical performance of the HPLC-UV-PCO-CV-AFS technique was evaluated using methylmercury and inorganic mercury standards. The retention times were 19 and 24 min for methylmercury and inorganic mercury, respectively. The detection limit of both mercury species, 10 ± 2 pg or 51 + 9 ng l^{-1}, was calculated from calibration curves constructed in the range 100–800 ng l^{-1} and based on the amount necessary to yield a net signal equal to three times the standard deviation of the blank. The relative standard deviation (n = 4) for a 300 ng l^{-1} methylmercury standard was < 1%.

Extraction procedure

Samples of 0.15 g were weighed into glass tubes, 6 ml of 25% m/v methanolic potassium hydroxide were added and the tubes were capped. The slurry was homogenized by magnetic agitation and submitted to microwave irradiation for 30–180 s at 48–132 W. The digestate was allowed to cool to laboratory temperature. The alkaline extract was mechanically shaken in a glass separating funnel for 10 min with 6 ml of dichloromethane and 4.5 ml of concentrated hydrochloric acid. With this procedure organomercury compounds were extracted into the organic phase, whereas inorganic mercury remained in the aqueous phase as chloro complexes. An aliquot of dichloro-

methane was transferred into another tube and the slurry was again treated with 6 ml of dichloromethane for 10 min. Finally, the dichloromethane layers were combined and organomercurials were solvent exchanged into 35 ml of ultra-pure water by evaporation of the organic solvent with a current of nitrogen. This final solution was injected into the HPLC-UV-PCO-CV-AFS system. Blanks and methylmercury standards were subjected to the same procedure in order to check for possible contamination and analyte losses or interconversion of species, respectively.

Results and discussion

The use of concentrated acids for methylmercury extraction from biological samples can cause evaporation losses, methylmercury degradation to inorganic mercury or insufficient dissolution of the sample leading to low recoveries for methylmercury. However, several procedures based on alkaline digestion were found to be effective for methylmercury extraction from biological tissues. Since several hours (3–24 h) have been reported[6,32,33] as the time required for dissolving the sample in alkaline solutions, other workers[11,23] considered the use of sonication to reduce the time required for alkaline digestion (2–3 h). Recently, microwave-assisted alkaline digestion methods permitted the quantitative recovery of methylmercury from biological tissues in a few minutes.[17,18,20] Most of the alkaline digestion procedures for dissolution of biological tissues were used in combination with GC coupled to different element specific detectors. In this work, methylmercury determination in purified alkaline extracts of biological tissues was carried out by HPLC-UV-PCO-CV-AFS, since this coupled system has not been reported previously, to our knowledge.

Methylmercury stability in a microwave field

The behaviour of methylmercury in alkaline solution under microwave irradiation was investigated. For this purpose, 6 ml of 25% m/v methanolic potassium hydroxide were spiked with 7.5 ng of methylmercury and divided into two portions. The first portion was microwave irradiated at an applied power of 48–132 W for 3 min and the second portion was not exposed to microwave irradiation. Although similar experiments have already been carried out,[31] the power and time ranges were different to those in this work. All experiments were performed in two replicates and each replicate was measured twice. The methylmercury recovery, calculated for each microwave power as the ratio of the methylmercury concentrations determined with and without microwave treatment, was >95% at all applied powers. Additionally, no inorganic mercury signal was encountered in either of the chromatograms obtained. The results showed that neither evaporation losses nor methylmercury decomposition to inorganic mercury were produced without a reflux condenser[17,18] in a domestic microwave oven. In consequence, 25% m/v methanolic potassium hydroxide can be considered as an appropriate medium for the microwave-assisted extraction of methylmercury from fish tissues.

Optimization of microwave-assisted extraction procedure

In this work a series of experiments were carried out to optimize the microwave-assisted alkaline digestion procedure in terms of quantitative methylmercury recovery and minimum exposure time, through the evaluation of the effect of parameters such as microwave power and exposure time. For this purpose, 0.15 g of a biological certified reference material, NRC TORT-2, was suspended in 6 ml of 25% m/v methanolic potassium hydroxide and submitted to microwave irradiation at an applied power ranging from 48 to 132 W for exposure times of between 30 and 180 s. All experiments were performed in two replicates and each replicate was measured twice. A six-point standard additions method was always used in the determination step in order to avoid possible matrix interferences. The effects of both parameters (exposure time and microwave power) on methylmercury recovery (calculated as the ratio of determined and certified concentrations of methylmercury), are shown in Fig. 1, where it can be observed that the methylmercury recovery increased with increase in applied power for short exposure times. For exposure times longer than 80 s, the methylmercury recovery decreased, probably owing to losses by evaporation. In consequence, the optimum conditions for the proposed procedure were an applied power of 80–90 W and an exposure time of 1 min.

In order to ensure better precision and reproducibility between replicates, the use of sample amounts >0.15 g is possible. However, in such a case, two factors must be considered: (i) the relation between sample amount and volume of methanolic potassium hydroxide must be kept constant and (ii) the alkaline extract must be diluted in the same relation to avoid the occurrence of violent reactions between potassium hydroxide and hydrochloric acid, excessive formation of potassium chloride (which makes the phase separation difficult) and high consumption of dichloromethane and hydrochloric acid during the clean-up of the alkaline extract.

Matrix effects in biological tissue extracts

In order to check the effects of interferences on methylmercury determination in the purified alkaline extracts of fish tissue by HPLC-UV-PCO-CV-AFS, the slopes of the calibration curves obtained by adding different amounts of an aqueous standard of methylmercury to either ultra-pure water or fish tissue extracts were statistically compared. A historical series of analytical values was plotted (Fig. 2), which included aqueous standards,

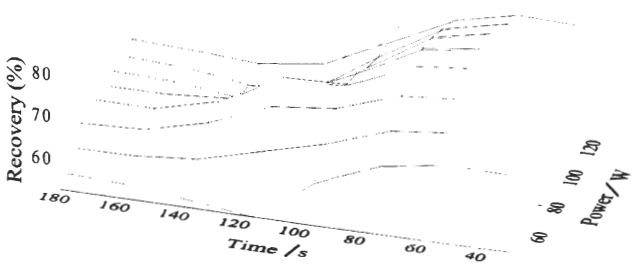

Fig. 1. Effect of microwave power and extraction time on methylmercury recovery from NRC TORT-2 biological reference material.

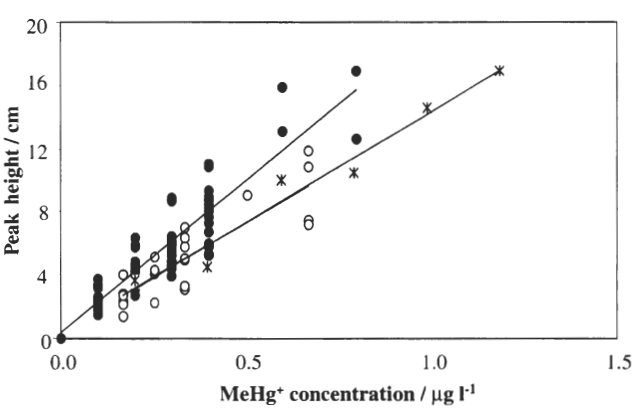

Fig. 2 Calibration curves for methylmercury obtained from aqueous standards (●) and spiked biological tissue extracts [NRC TORT-2 (○) and BCR 463 (*)].

Table 1 Slope and intercept values corresponding to the calibration curves for aqueous standards and biological tissue extracts spiked with methylmercury

Sample	Slope[34] $b \pm$ CL[a]/ cm l μg^{-1}	Intercept[34] $a \pm$ CL[b]/cm
Aqueous standard[c]	19.143 ± 2.036	0.388 ± 0.624
Aqueous standard (n = 75)[d]	20.245 ± 1.003	
NRC TORT-2 (n = 22)[d]	14.734 ± 1.622	
BCR 463 (n = 6)[d]	14.371 ± 1.373	

[a] CL = t ESD$_l\sqrt{\Sigma(x_i - \bar{x})^2}$ (linear model with intercept); CL = t ESD/ $\sqrt{\Sigma x^2}$ (linear model with zero intercept).

[b] CL = t ESD $\sqrt{[1/n + \bar{x}^2/\Sigma(x_i - \bar{x})^2]}$ where ESD = $\sqrt{}$(residual sum of squares/residual degrees of freedom).

[c] Model: $y = a + bx$. [d] Model: $y = bx$.

Table 2 Methylmercury determination in certified reference materials

Reference material	Certified value.[a] $\bar{x} \pm ts/\sqrt{n}$ / mg kg^{-1}	Measured value.[a,b] $\bar{x} \pm ts/\sqrt{n}$ / mg kg^{-1}
NRC TORT-2	0.152 ± 0.013	0.133 ± 0.026
BCR 463	3.04 ± 0.16	2.90 ± 0.47

[a] Average value ± confidence limit (p = 0.05). [b] n = 3.

solutions of two certified reference materials (NRC TORT-2 and BCR 463) spiked with methylmercury. The analytical signals obtained for methylmercury from the certified reference materials without addition were subtracted from each point on the respective curve and the resulting difference was attributed to the signal of the standard. Table 1 gives the slope and intercept values corresponding to calibration curves obtained from aqueous standards of methylmercury and fish tissue extracts spiked with methylmercury. It can be observed that the 95% confidence interval for the slope of the calibration curve from aqueous standards did not include the slope of calibration curves obtained with the spiked extracts of reference materials, demonstrating the existence of matrix effects. Further, the 95% confidence interval for the true intercept of the aqueous standards included zero. Therefore, a linear model passing through the origin was fitted to all three cases. The existence of matrix effects was not very strong but it seemed recommendable to use the standard additions method. Taking into account the need to use the standard additions method, the sample throughput of the methodology was around four samples during 6 h of operation.

Validation of the analytical method

The analytical performance of the overall method was evaluated by the analysis of two biological certified reference materials, NRC TORT-2 and BCR 463, which contain different concentrations of methylmercury. Both materials were analysed in triplicate using the optimum conditions established for the microwave-assisted alkaline digestion. The certified and the measured values of methylmercury concentration in both reference materials were statistically compared by an F-test for variances and a t-test for means and they were not statistically different at p = 0.05 (Table 2). Methylmercury concentrations determined by the proposed method were in good agreement with the certified concentrations of methylmercury in both certified reference materials.

Fig. 3 shows a typical chromatogram of a sample of NRC TORT-2 without addition and a sample of the same material spiked with 0.34 ng ml^{-1} of methylmercury standard. The

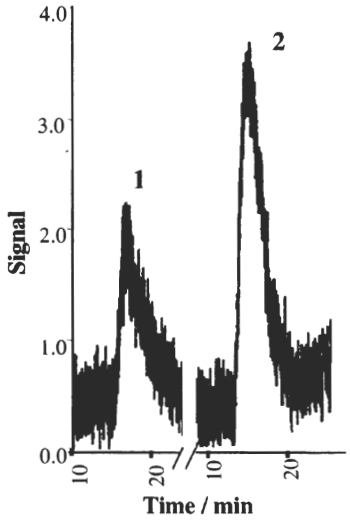

Fig. 3 Chromatogram of the NRC TORT-2 biological reference material. Peak 1, methylmercury; peak 2, methylmercury and standard addition of 0.34 ng ml^{-1} methylmercury standard.

relative standard deviation was <8% (n = 3). The detection limit of the method for methylmercury determination by HPLC-UV-PCO-CV-AFS was 10 μg kg^{-1}.

Conclusions

The proposed microwave-assisted extraction procedure shortened the time for alkaline digestion of biological tissues with 25% m/v methanolic potassium hydroxide from 3 h to 1 min in comparison with other work published previously,[6,32] and also achieved the quantitative recovery of methylmercury. Furthermore, this sample treatment procedure offers the possibility of conducting several simultaneous extractions. The alkaline extracts were analysed by HPLC-UV-PCO-CV-AFS and the overall method was validated through the analysis of two biological certified reference materials. The results obtained confirm the efficiency, reproducibility and accuracy of the proposed method. The detection limit obtained, 10 μg kg^{-1}, was similar to those in other work reported with the combination of alkaline digestion with GC coupled to AAS,[17,23] AFS[32] or AES.[35]

Acknowledgements

The authors acknowledge the help of Dr G. Millward in suggesting different water removal systems and to PS Analytical for helping us with the AFS detector. Two research grants are also gratefully acknowledged: a PhD grant (PRAXIS/BD/11153/97) to E. Ramalhosa and a Post-doctoral grant from the Xunta de Galicia to S. Río Segade.

References

1 P. J. Craig, in *Organometallic Compounds in the Environment–Principles and Reactions*, ed. P.J. Craig, Longman, Harlow, 1986, pp.65–101.
2 N. S. Bloom, *Can. J. Fish. Aquat. Sci.*, 1992, **49**, 1010.
3 Ph. Quevauviller, E. A. Maier and B. Griepink, in *Quality Assurance for Environmental Analysis*, ed. Ph. Quevauviller, E.A. Maier and B. Griepink, Elsevier, Amsterdam, 1995, pp. 1-25.
4 G. Westöö, *Acta Chem. Scand.*, 1967, **20**, 1790.
5 G. Westöö, *Acta Chem. Scand.*, 1968, **22**, 2277.

6 L. Liang, M. Horvat, E. Cernichiari, B. Gelein and S. Balogh, *Talanta*, 1996, **43**, 1883.
7 H. Emteborg, N. Hadgu and D. C. Baxter, *J. Anal. At. Spectrom.*, 1994, **9**, 297.
8 E. Bulska, D. C. Baxter and W. Frech, *Anal. Chim. Acta*, 1991, **12**, 545.
9 M. Horvat, N. S. Bloom and L. Liang, *Anal. Chim. Acta*, 1993, **281**, 135.
10 R. Falter and G. Ilgen, *Fresenius' J. Anal. Chem.*, 1997, **358**, 401.
11 Y. Cai and J. M. Bayona, *J. Chromatogr., A*, 1995, **696**, 113.
12 F. Palmisano, P. G. Zambonin and N. Cardellicchio, *Fresenius' J. Anal. Chem.*, 1993, **346**, 648.
13 R. Falter and H. F. Scholer, *Chemosphere*, 1994, **29**, 1333.
14 S. Río Segade and C. Bendicho, *J. Anal. At. Spectrom.*, 1999, **14**, 263.
15 W. Holak, *J. AOAC Int.*, 1995, **78**, 1124.
16 R. Cela-Torrijos, M. Miguens-Rodríguez, A. M. Carro-Díaz and R. A. Lorenzo-Ferreira, *J. Chromatogr., A*, 1996, **750**, 191.
17 C. M. Tseng, A. de Diego, F. M. Martin, D. Amouroux and O. F. X. Donard, *J. Anal. At. Spectrom.*, 1997, **12**, 743.
18 I. Rodríguez Pereiro, A. Wasik and R. Lobinski, *Anal. Chem.*, 1998, **70**, 4063.
19 C. F. Harrington, J. Romeril and T. Catterick, *Rapid Commun. Mass Spectrom.*, 1998, **12**, 911.
20 C. Gerbersmann, M. Heisterkamp, F. C. Adams and J. A. C. Broekaert, *Anal. Chim. Acta*, 1997, **350**, 273.
21 M. J. Vázquez, M. Abuín, A. M. Carro, R. A. Lorenzo and R. Cela, *Chemosphere*, 1999, **39**(7), 1211.
22 E. Bulska, D. C. Baxter and W. Frech, *Anal. Chim. Acta*, 1991, **249**, 545.
23 R. Fischer, S. Rapsomanikis and M. O. Andreae, *Anal. Chem.*, 1993, **65**, 763.
24 N. Bloom, *Can. J. Fish. Aquat. Sci.*, 1989, **46**, 1131.
25 H. Hintelmann and R. D. Evans, *Fresenius' J. Anal. Chem.*, 1997, **358**, 378.
26 H. Hintelmann, R. Falter, G. Ilgen and R. D. Evans, *Fresenius' J. Anal. Chem.*, 1997, **358**, 363.
27 I. Medina, E. Rubi, C. Mejuto, C. Casais and R. Cela, *Analusis*, 1993, **21**, 215.
28 R. Eiden, R. Falter, B. Agustin-Castro and H. F. Scholer, *Fresenius' J. Anal. Chem.*, 1997, **357**, 439.
29 R. Falter and G. Ilgen, *Fresenius' J. Anal. Chem.*, 1997, **358**, 407.
30 H. Hintelmann and R. D. Wilken, *Appl. Organomet. Chem.*, 1993, **7**, 173.
31 E. Ramalhosa, S. Río Segade, E. Pereira, C. Vale and A. Duarte, *J. Anal. At. Spectrom.*, 2001, **16**, 643.
32 E. Saouter and B. Blattmann, *Anal. Chem.*, 1994, **66**, 2031.
33 W. Baeyens, *Trends Anal. Chem.*, 1992, **11**, 245.
34 R. Caulcutt and R. Boddy, *Statistics for Analytical Chemists*, Chapman and Hall, London, 1983, pp. 80-83, 90-93.
35 M. S. Jimenez and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 1997, **12**, 597.